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Review

The neuroanatomy of sexual dimorphism in opioid analgesia

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ABSTRACT

The influence of sex has been neglected in clinical studies on pain and analgesia, with the vast majority of research conducted exclusively in males. However, both preclinical and clinical studies indicate that males and females differ in both the anatomical and physiological composition of central nervous system circuits that are involved in pain processing and analgesia. These differences influence not only the response to noxious stimuli, but also the ability of pharmacological agents to modify this response. Morphine is the most widely prescribed opiate for the alleviation of persistent pain in the clinic; however, it is becoming increasingly clear that morphine is less potent in women compared to men. This review highlights recent research identifying neuroanatomical and physiological dimorphisms underlying sex differences in pain and opioid analgesia, focusing on the endoge nous descending pain modulatory circuit.

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Introduction

Sex differences in pain and its control have long been a debated issue for scientists and healthcare providers hoping to optimize pain treatment for the individual. The recent drive towards evidence based medicine has further highlighted this issue as healthcare providers look to the research literature for making important decisions regarding pain treatment in the clinic. Recently, the Sex, Gender and Pain special interest group of the International Association for the Study of Pain (IASP) issued a consensus paper highlighting the need for inclusion of both males and females in pre clinical and clinical studies on pain and its management (Greenspan et al., 2007). This multidisciplinary consen sus was triggered by the need for application of basic science to clinical problems to continue to advance our understanding of how one's biological sex influences potential pain mechanisms and therapeutic strategies.

Sex differences in pain and morphine analgesia

Clinical studies on pain and analgesia are increasingly including sex (or gender) as an independent variable. Indeed, the number of studies examining sex differences in pain and analgesia has increased by 3500% since 1980 (Fillingim et al., 2009). Experimentally induced pain across a wide range of stimuli, including noxious pressure, electrical, ischemic, and thermal stimuli, form the majority of these studies. Measures of pain sensitivity include threshold and tolerance, and self report ratings of unpleasantness. For the most part, these studies

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consistently report that females display lower pain thresholds and decreased tolerance to noxious stimuli in comparison to men (Berkley, 1997; Mogil and Bailey, 2010). However, specific underlying mecha nism(s), including sex differences in hormone status, have yet to be identified. Positron emission tomography (PET) scanning studies have reported that experimental pain induces a larger magnitude of activa tion of the endogenous mu opioid system in males compared to females (Zubieta et al., 2002). Specifically, men demonstrated larger magnitudes of MOR activation than women in the anterior thalamus, ventral basal ganglia, and amygdala. Conversely, women showed reduced activation of the mu opioid system during pain in the nucleus accumbens. These data suggest that the magnitude, direction and site of activation of the endogenous opioid system is sex dependent and likely contributes to the increased pain sensitivity in females reported in pre clinical experimental pain studies.

While it is clear that females suffer from the majority of chronic pain syndromes, including fibromyalgia, temporomandibular syndrome, and irritable bowel syndrome (Cairns, 2007; M. Heitkemper and M. Jarrett, 2008; M.M. Heitkemper and M.E. Jarrett, 2008; Leresche, 2011; Mayer et al., 2004), studies assessing pain levels across sexes for similar ailments are more challenging to interpret (Cepeda and Carr, 2003; Sarton et al., 2000). A survey of studies examining sex differences in post operative and/or procedural pain (including outpatient surgery (Chia et al., 2002), knee arthroscopic repair (Rosseland and Stubhaug, 2004; Rosseland et al., 2008), and cholecystectomy (De Cosmo et al., 2008)) reported either no sex difference or greater sensitivity in females (Fillingim et al., 2009). Rarely is it reported that males display increased sensitivity.

Unfortunately, pre clinical and clinical studies examining sex differ ences in morphine analgesia are less consistent. Findings of greater analgesia in males versus females, females versus males, and no sex differences following opioid administration have all been reported (Cepeda and Carr, 2003; Fillingim et al., 2005; Gordon et al., 1995; Sarton et al., 2000). One complicating factor is that many of these studies were conducted in an experimental pain setting in which healthy volunteers rated the unpleasantness of a variety of acute noxious stimuli before and after morphine administration. Morphine is typically prescribed for the alleviation of a persistent and/or severe pain state, and it is clear that persistent pain alters the way the central nervous system (CNS) responds to opiates (Eidson and Murphy, 2013a, 2013b). Future studies using assays that more closely mimic the conditions for which morphine is prescribed may help clarify the impact of sex and/or gender on morphine's ability to elicit analgesia.

Retrospective studies examining the impact of sex on morphine consumption (including patient controlled analgesia) have reported that males typically consume more morphine than females for post surgical pain relief (Chia et al., 2002; Miaskowski et al., 2000). However, given that the side effects associated with morphine consumption, including nausea, headache and dysphoria, are exacerbated in females compared to males, morphine *consumption* may not be a reliable indica tor of morphine *analgesia* (Fillingim et al., 2005). A limited number of studies have examined the impact of sex on the pain relieving proper ties of morphine in a clinically relevant setting. Unfortunately, these results are far from consistent. Cepeda and Carr (2003) reported that fe males required 30% more morphine to reach the same level of analgesia as males. By contrast, Sarton *et al.* reported greater morphine analgesia in females (Sarton et al., 2000), while other studies reported no sex difference (Fillingim et al., 2005; Gordon et al., 1995).

Results from pre clinical behavioral models examining the impact of sex on opiate analgesia are more consistent, although opiate receptor specificity, route and dose of drug administration, type of analgesiometric test employed, and species and strain of animal tested have been shown to influence the pharmacodynamics of opiate analgesia (see Mogil, 2012 for review). Studies utilizing orofacial, somatosensory or visceral pain assays typically report that morphine produces a significantly greater degree of analgesia in male rodents compared to females (Craft, 2003; Craft et al., 2004; Ji et al., 2006; Loyd et al., 2008b; Wang et al., 2006). The reported sex differences in morphine analgesia are not trivial; in both persistent inflammatory pain (Wang et al., 2006) and visceral pain (Ji et al., 2006) models, the median effective dose (ED_{50}) for systemic morphine is 2 fold higher in females than in males. Importantly, sex differences in morphine analgesia are not due to dimorphisms in the pharmacokinetics of morphine in humans (Sarton et al., 2000) or rodents (Cicero et al., 1997), as no sex differences in morphine elimination rates, or brain or serum levels have been reported (Cicero et al., 1996, 1997). Rather, these studies suggest that there is something inherently different about how morphine acts within the CNS of males and females to alleviate persistent pain.

Neural correlate of sexually dimorphic pain and analgesia

The midbrain periaqueductal gray (PAG), and its descending projec tions to the rostral ventromedial medulla (RVM) and spinal cord, comprises an essential neural circuit for both endogenous and exoge nous opioid mediated analgesia (Fig. 1) (Basbaum and Fields, 1978, 1984; Basbaum et al., 1978; Behbehani and Fields, 1979; Behbehani and Pomeroy, 1978). Acute and persistent pain activates the PAG, resulting in the release of endogenous opiates and a reduction in pain sensitivity. PAG stimulation produced analgesia is opioid mediated, as administration of the opiate antagonist naloxone completely blocks its effects (Akil et al., 1976). Indeed, stimulation of the PAG induces a pro found analgesic state, such that invasive surgery can be performed in the absence of exogenously administered analgesia (Reynolds, 1969). In humans, electrical stimulation of the PAG is used to alleviate intracta ble pain (Green et al., 2010; Levy et al., 2010).

The PAG contains a high density of mu opioid receptor (MOR) containing neurons (Commons et al., 1999, 2000; Gutstein et al., 1998; Kalyuzhny et al., 1996; Mansour et al., 1986, 1987; Wang and Wessendorf, 2002) and microinjection of opiate antagonists into the PAG significantly attenuates the analgesic effects of systemic morphine (Bernal et al., 2007; Lane et al., 2005; Zambotti et al., 1982). Similarly, administration of morphine, or other mu opioid receptor agonists, into the PAG produces potent analgesia, which is blocked by central or systemic administration of naloxone (Jensen and Yaksh, 1986). Anatomical studies indicate that approximately 27 50% of PAG neurons projecting to the RVM are MOR+ (Commons et al., 2000; Wang and Wessendorf, 2002).

The descending PAG RVM spinal cord pathway has been character ized anatomically and physiologically in the majority of vertebrate spe cies known to date (Bandler and Tork, 1987; Bandler et al., 1991; Behbehani, 1995; Behbehani and Fields, 1979; Beitz, 1982; Beitz et al., 1983). Not surprisingly, these studies were conducted exclusively in males with the implicit assumption that CNS neural circuits subserving pain and analgesia were organized in a comparable manner in females. However, recent anatomical and physiological studies in the rat indicate that the PAG RVM circuit is sexually dimorphic in both its anatomical organization and in its activation during persistent inflammatory pain states (Loyd and Murphy, 2006; Loyd et al., 2007). Similarly, the ability of morphine to suppress noxious stimulus induced excitation of the PAG is also sexually dimorphic.

Using a variety of complementary anatomical techniques, we first examined if there were qualitative and/or quantitative differences in the neural projection from the PAG to the RVM in male and female rats. Consistent with previous anatomical studies (Beitz et al., 1983; van Bockstaele et al., 1991), we reported dense projections from the dorsomedial, lateral and ventrolateral PAG to the RVM in male and female rats, with no overall qualitative sex differences noted (Loyd and Murphy, 2006). Interestingly, while the overall distribution pattern of PAG RVM projection neurons was comparable for both sexes, significant quantitative differences were observed, such that the number of PAG RVM output neurons was significantly greater in

females across the entire rostrocaudal axis of PAG (Fig. 2). Indeed, the average number of retrogradely labeled cells across the rostrocaudal extent of the PAG was 33% greater in female compared to male rats. The most prominent sex difference in retrograde labeling was observed within the lateral and ventrolateral regions of the caudal PAG, an area known to contain a dense distribution of mu opioid receptors (Kalyuzhny et al., 1996; Wang and Wessendorf, 1999).

Inflammatory pain results in the activation of descending modulatory circuits (Morgan et al., 1991; Williams et al., 1995) and contributes to both hyperalgesia and analgesia. Using Fos expression as a marker for neural activation, we reported that inflammatory hyperalgesia, induced by intraplantar injection of the inflammatory agent Complete Freund's Adjuvant (CFA), resulted in extensive Fos expression throughout the rostrocaudal axis of PAG in both male and female rats (Loyd and Murphy, 2006). Importantly, activation of the PAG was comparable (both quantitatively and qualitatively) in male and female rats, and is consistent with our finding of no sex differences in the degree of hyperalgesia observed following intraplantar CFA (Loyd and Murphy, 2006). However, when the analysis of inflammatory pain induced Fos was restricted to PAG neurons with direct projections to the RVM, females showed very low levels of activation, despite having almost 2× as many PAG RVM neurons (Fig. 2). This suggests that inflammatory pain preferentially activates the PAG RVM circuit in males, but not females. Indeed, we found that, overall, persistent inflammatory pain activated approximately 43% of PAG RVM neurons in the dorsomedial, lateral and ventrolateral PAG of male, but only half as many in females. As activation of the PAG RVM pathway results in the inhibition of dorsal horn neuronal responses to noxious stimulation and suppresses pain, one would predict that given the greater activation of the PAG RVM circuit in males in comparison to females, males should have displayed reduced hyperalgesia following induction of hindpaw inflammation. However, this was not the case. Both males and females displayed similar levels of hyperalgesia following intraplantar CFA, suggesting an alternative (non PAG RVM) circuit for endogenous pain modulation in females.

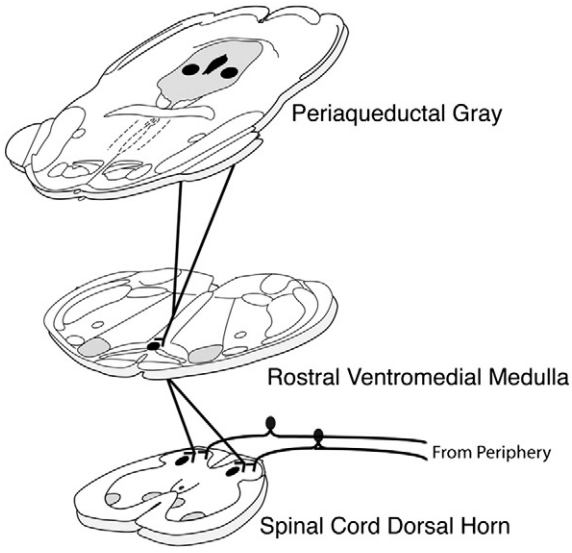

Fig. 1. A schematic of the descending inhibitory pathway for pain modulation illustrating the projections from the midbrain periaqueductal gray to the brainstem rostral ventromedial medulla and the spinal cord dorsal horn at the level of incoming stimulation from the periphery.

Sex differences in response to systemic morphine: role of the PAG–RVM circuit

In the majority of preclinical pain studies, morphine consistently produces a greater degree of analgesia in male compared with female rats, with similar, although not unequivocal effects observed in humans. As reviewed above, several lines of evidence indicate that the PAG is an essential locus for exogenous opioid mediated analgesia. In our previous studies, we reported that systemic morphine administration attenuated persistent inflammatory pain induced Fos in the PAG of male, but not female, rats (Loyd and Murphy, 2006), a finding consistent with studies reporting that the ED_{50} value for systemic morphine is approximately 2 fold higher in females compared to males (Chia et al., 2002). Interestingly, morphine administration, in the absence of pain, resulted in a 2 fold greater activation of PAG neurons in both males and females compared to saline administration (Loyd et al., 2007). No sex difference was observed in the activation of PAG neurons by morphine, suggesting that in the absence of pain, morphine is equipotent in its ability to depolarize PAG neurons. However, when analysis is limited to PAG RVM neurons, the number of neurons activated by morphine is consistently and significantly higher in males compared to females. Indeed, approximately half of the PAG RVM neurons in males were activated by morphine, whereas only 20% were activated in females.

In subsequent studies, we examined the role of the PAG RVM circuit in the development of morphine tolerance (Loyd et al., 2008a). Repeated administration of an ED_{50} dose of morphine induced tolerance in males to a significantly greater extent than in females. In parallel, morphine activation of PAG RVM neurons was significantly attenuated following repeated morphine administration in males. While no sex difference in the overall activation of the PAG was observed following 3 or 6 doses of morphine over 3 days (5 mg/kg; i.p.), the specific activation of the PAG RVM circuit by morphine steadily declined in males only. Morphine activation of this pathway in female rats was minimal, and therefore did not decline significantly following repeated administration of morphine. Together, these studies suggest that sex differences in morphine's ability to *engage* the PAG RVM pathway contributes its dimorphic pain relieving properties.

Direct administration of morphine or MOR selective agonists into the PAG also results in sex dependent analgesia. Krzanowska and Bodnar (Krzanowska and Bodnar, 1999b) reported intra PAG morphine ED_{50} values of 1.2 μg for male rats in comparison to >50 μg in estrus female rats. In a model of persistent inflammatory pain, we reported intra PAG morphine ED_{50} values for males of 7.5 μg versus 15 μg for females (Loyd et al., 2008b). The antinociceptive effects of morphine are mediated primarily by mu opioid receptors; therefore, our subsequent experiments tested the hypothesis that sex differences in MOR expression within the PAG contributed to our observed sex differences in morphine analgesia. Using both immunohistochemistry and autoradiography, we report that males have significantly higher levels of MOR expression and binding along the rostrocaudal axis of PAG (Fig. 2). Furthermore, we found that mu opioid receptor expressing PAG neurons appear to be necessary for eliciting the sexually dimorphic response to morphine as site directed lesions of mu opioid receptor expressing PAG neurons dose dependently reduced morphine analgesia in males only (Loyd et al., 2008b), making them similar to females in their response to morphine.

In addition to MOR, sex differences in the initiation of second messenger signaling cascades by morphine have also been reported (Burstein et al., 2013; Craft et al., 2001; Mitrovic et al., 2003; Schwindinger et al., 2009). Morphine post synaptically inhibits G protein coupled inwardly rectifying potassium channels (GIRKs) and sex differences in signal transduction of morphine by GIRK have been reported (Mitrovic et al., 2003). While wild type male mice exhibit higher pain thresholds and greater morphine analgesia than female mice, male mice lacking the GIRK2 channel subunit exhibit reduced pain thresholds and morphine analgesia levels similar to wild type females (Mitrovic et al., 2003). Altered signal transduction following activation of membrane estrogen receptors may also be involved in modulating analgesia in females.

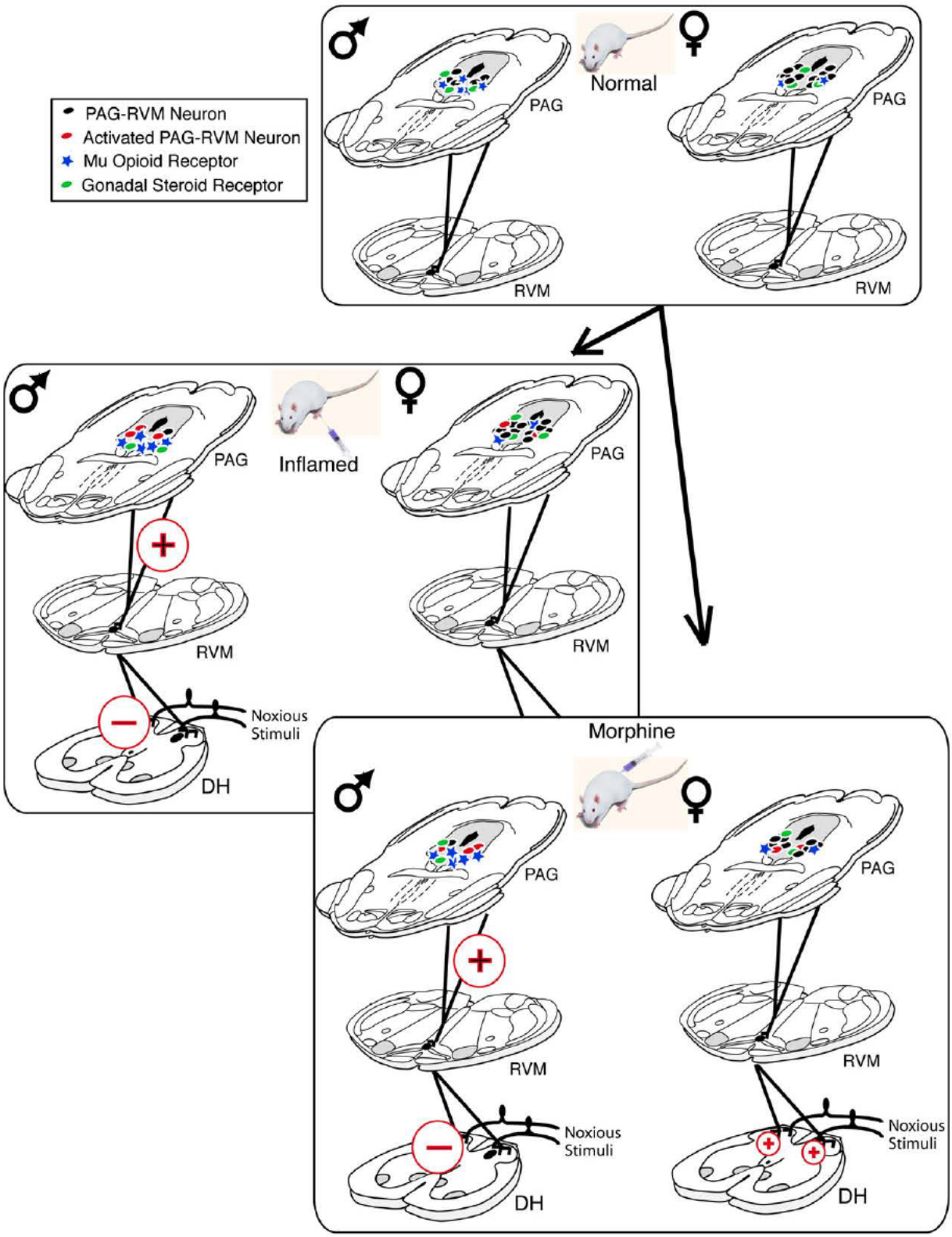

Fig. 2. Proposed model of factors contributing to sex differences in pain and morphine potency. Sex differences in the PAG–RVM projections, mu opioid receptors, and gonadal steroid receptors in the PAG (panel 1) are involved in modulating the activation of the PAG–RVM circuit to a differential degree in males and females to produce sex differences in endogenous pain inhibition under inflammatory conditions (panel 2) and systemic morphine administration (panel 3).

Role of gonadal hormones in sex differences in morphine analgesia

Sex differences in gonadal hormone concentrations appear to play a contributing role in sex differences in pain and analgesia (Stoffel et al., 2003). In women, there is evidence that pain fluctuates across the ovarian cycle, as well as during pregnancy and menopause (Berkley, 1997). Circulating levels of estradiol across the rat estrous cycle reportedly influence pain and morphine analgesia as well, with greater potency reported during diestrus, when circulating estradiol is lowest (Craft et al., 2004). An organizational effect of gonadal steroids is also

likely. For example, male rats feminized at birth demonstrate reduced morphine potency in adulthood, while masculinized female rats demonstrate greater morphine potency (Krzanowska and Bodnar, 1999a).

The PAG RVM circuit is an essential pathway by which morphine produces an analgesic response; therefore, we hypothesized that sex differences in the steroid regulation of the PAG RVM pathway may contribute to sex dependent pain thresholds or opioid analgesia. The PAG contains a large population of both estrogen (ERα) and androgen (AR) receptor containing neurons (Murphy and Hoffman, 1999, 2001). Indeed, this region contains the largest population of steroid receptors outside of the hypothalamus. Both ERα and AR immunoreactive neurons are localized primarily within the dorsomedial, lateral and ventrolateral regions of PAG (Loyd and Murphy, 2008). While the expression of ERα in the PAG is comparable between the sexes, males have a significantly greater number of AR immunoreactive neurons localized within the dorsomedial, lateral and ventrolateral PAG compared to females (Fig. 2) (Loyd and Murphy, 2008). AR binds 5,7 DHT, the 5α reduced metabolite of testosterone. Future studies manipulating 5,7 DHT levels are warranted to determine the role of increased PAG AR expression in morphine analgesia.

Approximately 30 37% of PAG neurons projecting to the RVM express AR or ERα, with the highest density of colocalization noted in the lateral/ventrolateral region of the caudal PAG. This PAG region also contains the highest density of MOR and suggests a direct mechanism whereby changes in endogenous gonadal steroid levels could modulate morphine analgesia. Consistent with previous studies, we found that the antinociceptive properties of intra PAG morphine were significantly reduced in female rats during both proestrus and estrus in comparison to diestrus (when estrogen and progesterone are lowest) (Islam et al., 1993; Kepler et al., 1989; Krzanowska and Bodnar, 1999b; Krzanowska et al., 2002). In fact, analgesia resulting from intra PAG morphine to diestrus females was not significantly different from males (Loyd et al., 2008b). These results parallel our findings of reduced MOR protein levels and binding during proestrus compared with diestrus, and provide further support that the amount of available MOR is positivity related to the degree of analgesia produced by morphine.

Estradiol has been shown to uncouple the mu opioid receptor from G protein gated inwardly rectifying potassium channels (Kelly et al., 2003), resulting in an attenuation of morphine induced hyperpolarization. Estradiol has also been shown to induce MOR internalization (Eckersell et al., 1998), thereby reducing available opioid binding sites on the cell membrane. Interestingly, ERα is required for estradiol induced MOR internalization (Micevych et al., 2003) supporting the hypothesis that colocalization of MOR and ERα in the PAG RVM output neurons provides a unique mechanism through which estrogens may differentially affect morphine potency in male and female rats (see Gintzler and Liu, 2012 for review).

Spinal antinociception is sexually dimorphic and dependent on gonadal hormones

In addition to the PAG, numerous studies suggest that sex differences in the anatomical, physiological and biochemical organization of the spinal cord also contribute to the dimorphic effects of opiates. The dorsal horn of the spinal cord is densely populated with MOR, and sex differences in analgesia can be elicited following intrathecal administration of either endogenous or exogenous opioid ligands. For instance, endomorphin, the predominant endogenous opioid ligand in the spinal cord, is more effective at producing spinal antinociception in male rats (Liu and Gintzler, 2013). This effect is hormone dependent. During diestrus, when circulating estrogens are low, spinal antinociception to endomorphin was minimal. In contrast, during proestrus, when circulating estrogens are high, spinal endomorphin antinociception was robust and comparable in magnitude to that noted in males.

Sex differences in the neuroendocrine organization of the spinal cord likely contribute to the dimorphic effects of morphine (Small et al., 2013). The spinal cord dorsal horn contains high levels of estrogen receptors (both ERα and ERβ; (Liu et al., 2007; Papka et al., 2001)), and there is evidence that these receptors interact with both MOR and KOR at the level of the spinal cord to alter antinociception (Gupta et al., 2007; Liu et al., 2013). Kappa opioid receptors form heterodimers with MOR (KOR/MOR) in the spinal cord (Chakrabarti et al., 2010), and the levels of KOR/MOR are approximately 4 fold greater in the spinal cord of proestrus female versus male rats. Sex differences in KOR/MOR hetero dimers contribute to the sexually dimorphic effects of intrathecal morphine such that in females, but not males, activation of spinal κ opioid receptors is a prerequisite for spinal morphine antinociception. Interestingly, activation of spinal kappa receptors alone does not induce antinociception, indicating the requirement for KOR/MOR dimer activation in morphine analgesia (Liu et al., 2007).

Changes in hormonal status have also been reported to influence peripheral pain processing (Fillingim and Ness, 2000; Flake et al., 2006; Gintzler, 1980; Gintzler and Bohan, 1990; Ji et al., 2003, 2005, 2007). For example, using a recently developed *in vitro* superfusion method to measure proinflammatory peptide release from human dental pulp from extracted teeth (Fehrenbacher et al., 2009), Loyd et al. (2012) reported sex differences in inflammation induced proinflammatory peptide release that was dependent on stage of menstrual cycle. Specifically, inflammatory mediator evoked proinflammatory peptide release was highest in amenstrual females and females in the last week of menses (Loyd et al., 2012). Changes in hormonal status have also been reported to contribute to a variety of pain disorders that are more common in women, including migraine, fibromyalgia and irritable bowel syndrome. Together, these data should be considered when assessing pain and providing pain therapy to women, especially in persistent pain disorders that involve an inflammatory component.

Implications on future research and pain management

Research spanning four decades has shown that the PAG, and its descending projections to the RVM and spinal cord dorsal horn, constitute an essential neural circuit for opioid based analgesia. During the last half of that period, numerous rodent and human studies have established sex differences in pain and the analgesic effects of morphine at each level of this circuit. Sex differences in pain and morphine analgesia are likely due to the inherent differences in how the central nervous system responds to pain and opioid based analgesia. The anatomical, physiological and biochemical properties by which morphine produces analgesia are sexually dimorphic in the PAG and spinal cord, with clear biological consequences in terms of pain modulation and morphine action. Current research suggests that morphine may not be the drug of choice for pain management in women; thus, research efforts need to be devoted toward the identification of more effective pain therapeutics for the management of persistent pain in women.

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